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Cellular focal segmental glomerulosclerosis: Clinical and pathologic features

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Five pathologic variants of idiopathic focal segmental glomerulosclerosis (FSGS) are recognized: collapsing (COLL), cellular (CELL), glomerular tip lesion (GTL), perihilar, and not otherwise specified (NOS). The prognostic significance of CELL FSGS has not been determined. We compared the presenting clinical and pathologic characteristics in 225 patients with CELL ($N=22$), COLL ($N=56$), GTL ($N=60$), and NOS ($N=87$) variants of idiopathic FSGS. CELL, COLL, and tip lesion all showed greater frequency and severity of nephrotic syndrome, and shorter time to biopsy compared to NOS. Predictors of end-stage renal disease (ESRD) for all FSGS patients included initial serum creatinine, % global sclerosis, % COLL lesions, chronic tubulo-interstitial injury score, and lack of remission response. COLL FSGS had the highest rate of renal insufficiency at presentation, most extensive glomerular involvement and chronic tubulo-interstitial disease, fewest remissions (13.2%), and highest rate of ESRD (65.3%). GTL patients were older and showed the highest remission rate (75.8%) and lowest rate of ESRD (5.7%). CELL variant showed intermediate rates of remission (44.5%) and ESRD (27.8%) compared to COLL and tip lesion. CELL variant may include cases of unsampled tip or COLL lesion, underscoring the importance of adequate sampling. Our data support the view that CELL and COLL FSGS are not equivalent and validates an approach to pathologic classification that distinguishes between COLL, CELL, and tip lesion variants of FSGS.

Kidney International (2006) **70,** 1783–1792. doi:10.1038/sj.ki.5001903; published online 4 October 2006

KEYWORDS: renal biopsy; renal pathology; focal segmental glomerulosclerosis; histopathology; idiopathic nephrotic syndrome

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Received 4 March 2006; revised 24 July 2006; accepted 15 August 2006; published online 4 October 2006

A pattern of focal segmental glomerulosclerosis (FSGS) may result from diverse pathogenetic mechanisms including heritable mutations of podocyte-specific proteins, infections, drug toxicities, and adaptive responses to reduced functioning renal mass.[1] For most patients with FSGS who present with nephrotic syndrome or heavy proteinuria, no secondary cause is identified (idiopathic FSGS). Idiopathic FSGS is clinically and pathologically heterogeneous and displays variable renal outcomes. Remission of nephrotic syndrome is the single most important predictor of long-term renal survival,[2–6] but morphologic classification of FSGS variants may provide additional prognostic information.[2]

The Columbia FSGS Classification employs a step-wise, hierarchical approach to distinguish five mutually exclusive variants of FSGS: collapsing (COLL), glomerular tip lesion (GTL), cellular (CELL), perihilar (PH), and not otherwise specified (NOS) (Figures 1–7).[1] The prognostic value of morphologic classification of idiopathic FSGS is supported by the observation that COLL FSGS has a more aggressive clinical course, with fewer remissions and more frequent end-stage renal disease (ESRD)[2,3] whereas GTL identifies a subset of FSGS that usually responds to steroids and rarely progresses to ESRD.[2,4] In the Columbia FSGS Classification, CELL is defined by presence of at least one glomerulus with segmental expansion of the glomerular tuft by endocapillary hypercellularity, often with foam cells, with or without hyperplasia of overlying visceral epithelial cells. Importantly, similar lesions may exist in COLL and GTL. Therefore, the diagnosis of CELL variant requires prior exclusion of any case with features of segmental capillary collapse (COLL), or with confluence of the CELL lesion with the origin of the proximal tubule (GTL). Using this classification schema, CELL is the least common variant of FSGS, identified in only 3% of cases of adult idiopathic FSGS in a recent study; the low number of CELL cases ($n=6$) in that study precluded statistical comparison of outcomes with other FSGS subgroups.[2] The relationship of CELL to COLL FSGS is controversial and some investigators do not distinguish between these subtypes.[5] Thus, the prognostic significance of CELL FSGS is undetermined.

In this study, we tested the hypothesis that CELL represents a distinct clinical–pathologic variant of

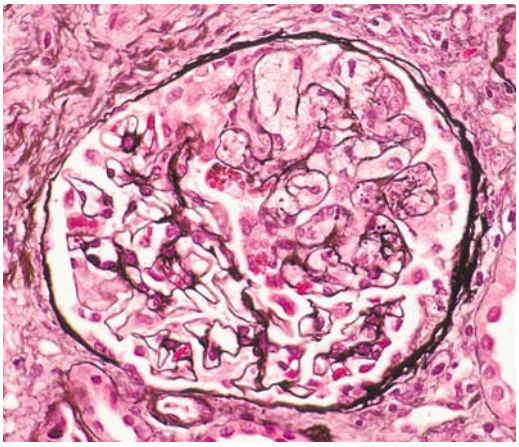

Figure 1 | CELL FSGS showing segmental obliteration of the glomerular capillary lumina by increased number of endocapillary cells, including endocapillary foam cells. Overlying podocytes are swollen and contain focal intracytoplasmic protein droplets. Features of capillary collapse or GTL are not seen. The afferent arteriole is identified at bottom (Jones silver methenamine stain; original magnification $\times 400$).

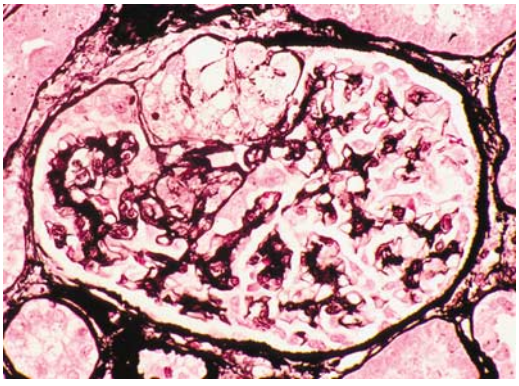

Figure 2 | CELL FSGS showing segmental engorgement and occlusion of glomerular capillary lumina by endocapillary foam cells, producing an expansile hypercellular lesion. There is a small adhesion to Bowman's capsule. No collapse is identified. The vascular and tubular poles are not identified in this plane of section (Jones silver methenamine stain; original magnification $\times 400$).

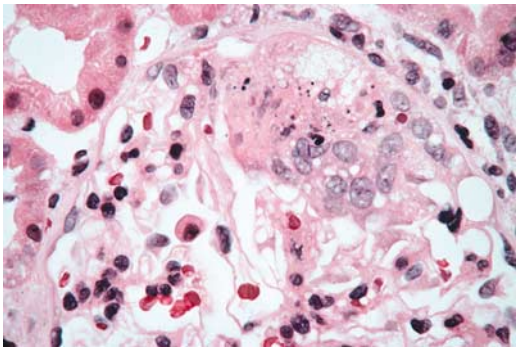

Figure 3 | CELL FSGS showing segmental endocapillary accumulation of foam cells and leukocytes, some of which are undergoing karyorrhexis. Features of capillary collapse or GTL are not seen. The afferent arteriole and proximal tubule are not visualized at this magnification (hematoxylin and eosin stain; original magnification $\times 600$).

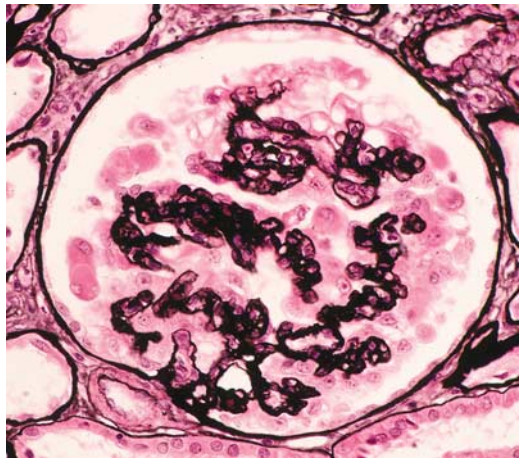

Figure 4 | COLL FSGS showing global obliteration of glomerular capillaries by implosive collapse of glomerular basement membranes and prominent hypertrophy and hyperplasia of the overlying podocytes, some of which are detached from the tuft (Jones silver methenamine stain; original magnification $\times 400$).

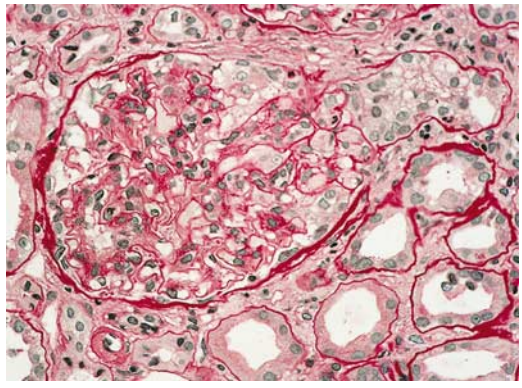

Figure 5 | Glomerular tip lesion. There is a segmental accumulation of endocapillary foam cells involving the peripheral glomerular segment at the tubular pole, adjacent to the origin of the proximal tubule. Podocytes overlying the segmental lesion show confluence with proximal tubular epithelial cells. Features of capillary collapse are not seen. The incoming arteriole is seen at the opposite pole (PAS reaction; original magnification $\times 250$).

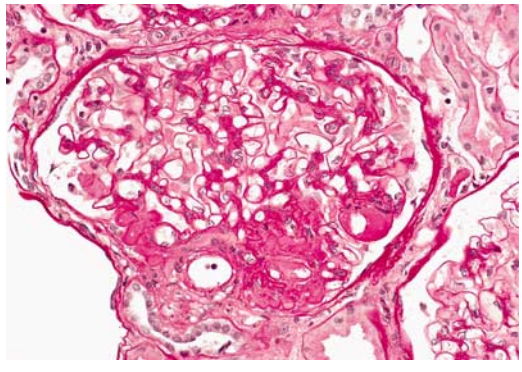

Figure 6 | PH FSGS. There is segmental occlusion of glomerular capillaries by matrix accumulation and hyalinosis at the glomerular hilus, identified by the incoming afferent arteriole and macula densa. Features of collapse, GTL, or endocapillary hypercellularity are not seen (PAS reaction; original magnification $\times 250$).

idiopathic FSGS by comparing the clinical and pathologic characteristics in a large cohort of patients with either CELL, COLL, GTL, or NOS in accordance with the Columbia FSGS Classification.[1]

RESULTS

Clinical characteristics of CELL and comparison to other FSGS variants

A total of 225 patients with idiopathic FSGS were studied, including 22 with CELL, 56 with COLL, 60 with GTL, and 87 with NOS. Demographic characteristics and presenting clinical features are summarized in Table 1. The cohort of CELL included 13 males and nine females. The mean age (±standard error of the mean) of CELL patients was 48.8±4.9 years and there were two children (ages 9 and 15 years). Seven patients were African-Americans, 12 were Caucasians, one was non-African American Hispanic, and two were Asians. Twenty-one CELL patients had nephrotic range proteinuria (NRP) (mean urinary protein excretion (UVp) 9.5±1.2 g/day) and 19 patients (86.4%) fulfilled all three defining criteria for the nephrotic syndrome (NRP + hypoalbuminemia + peripheral edema). Two subjects had NRP (3.8 and 4.0 g/day, respectively) but lacked evidence of edema or hypoalbuminemia and one subject had 2.5 g/day proteinuria, edema, and normal serum albumin (3.6 g/dl). The mean duration of symptoms before biopsy was 4.3±0.9 months. In 12 of 14 cases where the onset of symptoms was described, this was noted as acute, sudden, recent, or explosive. The mean serum albumin was 2.1±0.2 g/dl and mean serum cholesterol was 235.9±49.1 mg/dl. Fifteen patients (68.2%) had hypertension at the time of biopsy that was longstanding in nine cases and newly diagnosed in six. None of the remaining seven CELL patients who were normotensive were receiving antihypertensive medications. At presentation, 12 patients had renal insufficiency, including one who presented with ESRD, nine had normal renal function, and renal function data were not available for one individual. Five CELL subjects had acute renal insufficiency, based on normal renal function

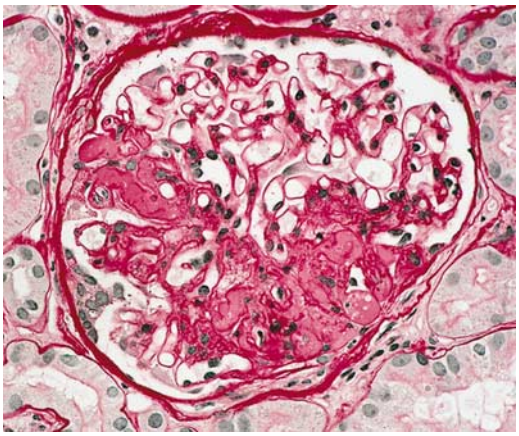

Figure 7 | FSGS lesion, NOS. There is segmental obliteration of capillary lumina by accumulation of matrix and hyaline. Features of collapse, GTL, or endocapillary hypercellularity are not seen. The tubular and vascular poles are not present in the plane of section (PAS reaction; original magnification ×400).

Table 1 | Baseline clinical characteristics of FSGS patients

Variable	All (*N*=225)	CELL (*N*=22)	COLL (*N*=56)	GTL (*N*=60)	NOS (*N*=87)	*P*=
Age (years)	37.7±1.3	48.8±4.9	31.2±2.3	47.5±2.3	32.5±2.0	0.002[A] 0.004[C] <0.001[D,F]
Male	48.9%	59.1%	46.4%	53.3%	44.8%	NS
Black	31.5%	31.8%	53.6%	13.8%	28.9%	<0.001[D] 0.003[E] 0.044[F]
Time to Bx (months)	14.2±2.9	4.3±0.9	10.0±2.8	2.1±0.3	45.2±10.9	0.005[C] 0.045[D] 0.027[E] 0.002[F]
UVp (g/day)	7.2±0.5	9.5±1.2	8.8±1.3	7.8±0.6	5.1±0.5	0.033[F]
Albumin (g/dl)	2.4±0.1	2.1±0.2	2.1±0.2	2.1±0.1	3.0±0.1	0.017[C] 0.001[E] <0.001[F]
Edema *N* (%)	161/213 (75.6)	20/22 (90.9)	46/54 (85.2)	54/57 (94.7)	41/85 (48.2)	<0.001[C,E,F]
Cholesterol (mg/dl)	263.8±13.7	235.9±49.1	297.0±32.5	223.3±26.3	272.1±18.7	NS
Nephrotic syndrome (%)	166/220 (75.5)	19/22 (86.4)	47/55 (85.5)	55/58 (94.8)	45/85 (52.9)	0.003[C] <0.001[E,F]
Creatinine (mg/dl)	2.3±0.2	1.9±0.3	3.9±0.6	1.5±0.1	1.9±0.2	0.026[A] 0.002[D] 0.012[E]
Creatinine > 1.2 mg/dl (%)	115/215 (53.5)	12/21 (57.1)	39/51 (76.5)	23/58 (39.7)	41/85 (48.2)	<0.001[D] 0.001[E]
HTN (%)	130/206 (63.1)	15/22 (68.2)	33/49 (67.4)	36/57 (63.2)	46/78 (59.0)	NS

Bx, biopsy; CELL, cellular FSGS; COLL, collapsing FSGS; GTL, glomerular tip lesion variant FSGS; NOS, FSGS, not otherwise specified; HTN, hypertension (including those with normal blood pressure receiving anti-hypertensive medication); *N*, number; NA, not available; NS, not significant; UVp, urinary protein excretion.
Quantitative variables are mean±standard error of mean.
A, CELL vs COLL; B, CELL vs GTL; C, CELL vs NOS; D, COLL vs GTL; E, COLL vs NOS; F, GTL vs NOS.

tests in the recent past; baseline renal status of the other seven individuals with renal insufficiency was unknown.

CELL and GTL patients were significantly older than COLL and NOS (all $P<0.005$). COLL showed a significantly higher proportion of black patients compared to GTL and NOS (both $P<0.005$) but not compared to CELL. NOS also showed significantly higher percentage of black patients than GTL. CELL, COLL, and GTL all showed significantly shorter time to biopsy than NOS (GTL < CELL < COLL < NOS). However, the difference between CELL and COLL (4.3 ± 0.9 vs 10.0 ± 2.8 months) did not reach statistical significance. Among 220 cases of idiopathic FSGS with sufficient clinical data, 75.5% of patients presented with the nephrotic syndrome. The mean UVp for all patients was 7.2 ± 0.5 g/day, mean serum albumin was 2.4 ± 0.1 g/dl, and mean serum creatinine was 2.3 ± 0.2 mg/dl. CELL, COLL, and GTL all showed a greater frequency of nephrotic syndrome and lower serum albumin than NOS, but there was no significant difference between CELL, COLL, and GTL. Cholesterol levels were similar in all four groups. COLL showed significantly higher initial serum creatinine than CELL, GTL, and NOS, and more frequent renal insufficiency (serum creatinine > 1.2 mg/dl) than GTL or NOS. The frequency of hypertension was similar in all four histologic subgroups.

Pathologic findings in CELL and comparison to other FSGS variants

Renal pathologic findings in CELL and the other FSGS variants are presented in Table 2. By light microscopy, the total number of glomeruli in CELL biopsies ranged from 7 to 26 (mean 14.6 ± 1.3). The percentage of globally sclerotic glomeruli in CELL cases ranged from 0 to 47% (mean $13.6\pm3.0\%$) and the percentage of segmental lesions per biopsy ranged from 4 to 30% (mean $17.1\pm2.9\%$). Most CELL biopsies showed mild tubulo-interstitial scarring (mean score 1.1, scale 0–3). No tubular microcysts were seen. Features of acute tubular injury were present in six biopsies, all from patients with renal insufficiency. Arteriosclerosis was present in 13 biopsies and this was mild (1+) in nine and moderate (2+) in four (mean score 0.8 ± 0.2).

Immunofluorescence microscopy in all 22 CELL biopsies was either completely negative or showed segmental glomerular staining for IgM and/or C3 consistent with nonspecific trapping.[6] Electron microscopy (EM) was performed in 19 cases. EM showed 95–100% foot process effacement in 18 cases and 60% effacement in one case. No endothelial tubuloreticular inclusions or structural glomerular basement membrane abnormalities were identified.

By definition, all CELL biopsies contained at least one CELL segmental lesion (range 1–4 per biopsy, mean 2) (Figures 1–3) and none showed features of COLL (Figure 4) or GTL (Figure 5). CELL lesions were generally small, involving 25–50% of the glomerular tuft. In 11 cases, CELL lesions contained foam cells (Figures 1 and 2); in the other 11 cases, CELL lesions contained mononuclear cells or neutrophils some of which were undergoing pyknosis or karyorrhexis (Figure 3). None of these lesions showed fibrinoid necrosis. Five of the 11 cases with foam cells also contained infiltrating mononuclear cells or neutrophils. All 22 CELL cases displayed mild ($n=13$) or moderate ($n=9$) visceral epithelial cell hypertrophy and hyperplasia overlying at least one CELL lesion (Figure 1) but not every CELL lesion had this feature. Twenty cases contained CELL lesions whose relationship to the glomerular hilus and proximal tubule was indeterminate. Peripheral CELL lesions were seen in five cases, three of which also showed CELL lesions of indeterminate location in other glomeruli and two of which showed only peripheral lesions. Importantly, none of these peripheral CELL lesions showed features of GTL (Figure 5) in adjacent serial sections. No PH lesions (Figure 6) were seen in

Table 2 | Comparison of pathologic features of FSGS cases

	All (153)	CELL (22)	COLL (46)	GTL (49)	NOS (36)	P=
# Glomeruli	26.3±2.4	14.6±1.3	33.8±5.0	25.1±2.9	26.3±6.9	0.005[A] 0.017[B]
% Global sclerosis	15.6±1.6	13.6±3.0	23.8±3.6	7.8±2.0	17.0±3.6	0.001[D]
% Collapsing	5.7±1.0	0	26.6±3.2	0	0	NA
% Cellular	5.1±0.6	14.0±1.6	1.5±0.6	12.3±1.4[a]	0	<0.001[A,D]
% Perihilar	1.8±0.4	0	2.4±0.9	0.1±0.1	4.9±2.4	0.003[C], 0.005[F]
% NOS	14.9±1.0	3.1±1.3	5.6±1.2	3.9±0.9	24.9±3.1	<0.001[C,E,F]
% Global sclerosis+% segmental lesions[b]	43.0±2.3	31.4±3.4	61.9±3.7	26.1±2.7	46.8±4.7	<0.001[A,D] 0.003[F]
TA/IF score (0–3+)	1.3±0.1	1.1±0.1	1.9±0.1	0.7±0.1	1.3±0.2	<0.001[A,D,E] 0.001[F]
Arteriosclerosis	0.9±0.1	0.8±0.2	1.1±0.1	0.7±0.1	1.0±0.1	NS
% FP fusion	84.2±2.0	95.6±2.2	76.4±4.2	93.6±1.4	70.9±5.1	0.001[A] 0.002[C,D,F]

CELL, cellular FSGS; COLL, collapsing FSGS; FP, foot process; GTL, glomerular tip lesion variant FSGS; IF, immunofluorescence microscopy; NOS, FSGS, not otherwise specified; *N*, number; NA, not applicable; TA/IF, tubular atrophy and interstitial fibrosis.
Variables are mean ± standard error of mean.
A, CELL vs COLL; B, CELL vs GTL; C, CELL vs NOS; D, COLL vs GTL; E, COLL vs NOS; F, GTL vs NOS.
[a]Mostly GTL.
[b]Segmental lesions include all collapsing, cellular, perihilar or NOS lesions.

any case of primary CELL. Parenthetically, PH sclerosis had been seen in three of the six cases of secondary CELL excluded on the initial review. Seventeen CELL cases (77.3%) showed only CELL lesions whereas five biopsies (22.7%) showed both CELL and NOS lesions (Figure 7); the percentage of segmental lesions that were NOS in these five biopsies was 33, 50, 60, 60, and 75%, respectively.

The total number of glomeruli in CELL cases (14.6 ± 1.3) was significantly less than COLL (33.8 ± 5.0, $P = 0.005$) or GTL (25.1 ± 2.9, $P = 0.017$), but not compared to NOS (26.3 ± 6.9, $P =$ NS). The five CELL cases with NOS lesions had higher % total segmental lesions than the 17 CELL cases without NOS lesions (25.6 ± 3.5 vs $15.8 \pm 3.1\%$, $P < 0.001$). However, there was no significant difference in the % CELL lesions between these two groups ($12.11 \pm 3.1\%$ with NOS lesions vs $15.8 \pm 3.1\%$ without NOS lesions). COLL showed significantly greater % global sclerosis than GTL (23.8 ± 3.6 vs $7.8 \pm 2.0\%$, $P = 0.001$) and significantly more segmental lesions of any type (38.1 ± 3.0) than CELL (17.1 ± 2.9, $P < 0.001$) or GTL (18.3 ± 1.8, $P < 0.001$). COLL showed significantly greater total glomerular involvement (global + segmental lesions) ($61.9 \pm 3.7\%$) than CELL ($31.4 \pm 3.4\%$, $P < 0.001$) and GTL ($26.1 \pm 2.7\%$, $P < 0.001$). Compared to NOS, GTL showed significantly fewer segmental lesions ($P < 0.047$) and fewer total lesions (global + segmental) ($P = 0.003$).

CELL showed significantly greater % cellular lesions (14.0 ± 1.6) than COLL ($1.5 \pm 0.6\%$) ($P < 0.001$), but not compared to GTL ($12.3 \pm 1.4\%$, $P =$ NS). CELL and GTL showed similar % global sclerosis (13.6 ± 3.0 vs $7.8 \pm 2.0\%$), % all segmental lesions (17.1 ± 2.9 vs $18.3 \pm 1.8\%$), % NOS lesions (3.1 ± 1.3 vs $3.9 \pm 0.9\%$), tubular atrophy/interstitial fibrosis score, arteriosclerosis score, and % foot process effacement (all $P =$ NS). NOS showed significantly more NOS lesions ($24.9 \pm 3.1\%$) than any other group (all $P < 0.001$). Most segmental lesions in GTL (including tip lesions) were cellular (mean 84% of all segmental lesions); the remaining lesions were sclerosing tip lesions, NOS, or PH lesions. PH lesions (Figure 6) were encountered in 10/46 COLL cases, 1/49 GTL cases, and 14/36 cases of NOS; in none of these cases did PH constitute $>50\%$ of segmental lesions. NOS cases had higher mean % PH lesions ($4.9 \pm 2.4\%$) than CELL (0%, $P = 0.003$) and GTL ($0.1 \pm 0.1\%$, $P = 0.005$). COLL showed significantly more severe chronic tubulo-interstitial injury than all other variants (all $P \leqslant 0.001$) and GTL showed significantly less chronic tubulo-interstitial injury than NOS ($P = 0.001$). All four subtypes showed similar arteriosclerosis score. Both CELL and GTL showed significantly higher % foot process effacement than COLL and NOS, but not compared to each other (95.6 ± 2.2 vs $93.6 \pm 1.4\%$, $P =$ NS).

Treatment and outcomes for CELL and comparison to other FSGS variants

Follow-up data were available for 187 of 225 FSGS patients (83.1%) including 18 CELL, 53 COLL, 33 GTL, and 83 NOS. Most patients (72.4%) received steroid therapy; however, details about duration and dose were not available in many cases. The majority of patients (51.3%) also received angiotensin-converting enzyme inhibitor (ACE) and/or angiotensin receptor blocker (ARB). After a median follow-up of 20.0 months, 38.5% of all FSGS patients had either complete or partial remission of nephrotic syndrome and 30.7% progressed to ESRD (Table 3).

Three CELL patients were lost to follow-up shortly after the biopsy and one died of a myocardial infarction before therapy for renal disease was initiated. The mean length of follow-up for the remaining 18 CELL patients was 17.4 ± 3.3 months (median 15.5 months, range 2–49 months). Sixteen patients received treatment for their renal disease including 14 who received steroids (eight of whom also received ACE and/or ARB) and two who received ACE/ARB alone. Among the patients treated with steroids: four also received sequential therapy with cyclosporine A, including one who later received cyclophosphamide; and two also received mycophenolate mofetil, followed by tacrolimus in one individual. Two CELL patients did not receive therapy, including one who presented with ESRD and one subject who was diagnosed with metastatic cancer and cirrhosis around the time of biopsy; the latter patient had no remission (NR) at 5 months follow-up.

Following therapy, seven CELL patients entered complete remission (CR) (including one individual who subsequently relapsed and had 2.8 g/day proteinuria and stable renal function at last follow-up). All seven CR patients received steroids; three also received ACE/ARB; and two received calcineurin inhibitor, including one who also received mycophenolate mofetil. Of note, two CR patients had mild renal insufficiency at presentation (serum creatinine 1.6 and 1.9 mg/dl, respectively) that recovered fully at last follow-up.

Ten CELL subjects were classified as NR, including one individual who presented with ESRD and one patient who showed significant reduction in UVp (from 14 to 0.9 g/day) but who had progressive renal insufficiency at last follow-up (serum creatinine increased from 1.5 to 2.7 mg/dl). The remaining eight NR patients all had persistent heavy proteinuria (urinary protein/creatinine ratio >2). Excluding the individual who presented with ESRD, the mean initial serum creatinine in the other nine NR patients was 2.2 mg/dl (range 0.9–6.3 mg/dl) and the mean final serum creatinine of those not progressing to ESRD was 2.1 mg/dl (range 0.9–3.7 mg/dl). Five NR subjects received steroids, including four with ACE/ARB, three with sequential cyclosporine A, and one each with sequential cyclophosphamide or sequential mycophenolate mofetil. One NR patient was treated with ACE/ARB alone. Two NR patients had normal renal function and heavy proteinuria at last follow-up. In addition to the one subject who presented with ESRD, four others developed ESRD (after 8, 10, 28, 35 months, respectively). One of these four subjects had normal renal function at presentation, two had renal insufficiency, and initial renal function data for the other individual was unknown. UVp in NR patients

Table 3 | Treatment and outcomes of FSGS cases

	All	CELL	COL	GTL	NOS	P=
N with follow-up	187	18	53	33	83	
Mean follow-up (months) (median)	33.7±2.7 (20.0)	17.4±3.3 (15.5)	25.7±4.8 (16.0)	21.4±4.3 (12.0)	46.3±4.7 (32.0)	<0.001[C] 0.017[E] 0.001[F]
Therapy						
Steroids	72.4%	77.8%	66.0%	97.2%	63.0%	0.038[B] <0.001[D,F]
ACE/ARB (% yes)	51.3%	55.6%	31.0%	66.7%	49.3%	0.042[A] 0.006[D]
CNI	19.5%	27.8%	9.3%	16.7%	30.6%	0.022[E]
Cytotoxics	17.9%	11.1%	19.2%	13.9%	20.9%	NS
MMF	1.3%	11.1%	0.0%	0.0%	0.0%	NS
Last sCr (mg/dl)	2.4±0.2	1.9±0.6	3.7±0.8	1.2±0.1	2.6±0.3	0.029[D] 0.002[F]
Last UPC	2.5±0.4	3.1±1.3	3.9±0.8	2.3±0.8	2.1±0.4	NS
Response						
CR:PR:NR	51:21:115	7:1:10	4:3:46	20:5:8	20:12:51	0.006[A] 0.086[B] <0.001[D,F] 0.005[E]
% CR+PR	38.5	44.4	13.2	75.8	38.6	0.009[A] 0.027[B] <0.001[D,F] 0.001[E]
% ESRD	30.7	27.8	65.3	5.7	34.5	0.023[A] 0.011[B] <0.001[D,F] 0.01[E]
Kaplan–Meier months survival to ESRD median	72±10	35±6	23±11	(85±8)[a]	90±10	0.211[A] 0.011[B] 0.0216[C] 0.0001[D] <0.0001[E]

ACE, angiotensin-converting enzyme inhibitor; ARB, angiotensin receptor blocker; Bx, biopsy; CELL, cellular FSGS; CNI, calcineurin inhibitors; COLL, collapsing FSGS; CR, complete remission; ESRD, end-stage renal disease; GTL, glomerular tip lesion variant; MMF, mycophenolate mofetil; NOS, FSGS, not otherwise specified; *N*, number; NA, not available; NC, not calculable; ND, not done; NR, no remission; NS, not significant; PR, partial remission; sCr, serum creatinine; UPC, urine protein/creatinine ratio.
Categorical variables are % affected; quantitative variables are mean±s.e.m., except where otherwise stated.
A, CELL vs COLL; B, CELL vs GTL; C, CELL vs NOS; D, COLL vs GTL; E, COLL vs NOS; F, GTL vs NOS.
[a]Mean value for GTL provided (median not calculable).

decreased from 8.9±1.9 to 6.2±2.3 g/day at last-follow-up (P = NS). One patient (who was treated with ACE only) had partial remission (PR) and stable renal function at last follow-up (UVp 0.4 g/day). At last follow-up, four of the 11 subjects with either NR or PR had stable renal function (mean follow-up 16.5±7.5 months, range 1–49 months); two had worsening renal function (at 23 and 28 months, respectively); and five were on dialysis.

Of the 12 CELL patients with renal insufficiency at time of biopsy: one was lost to follow-up; one immediately started dialysis (ESRD) and six had NR at last follow-up, including two who progressed to ESRD. The remaining four patients who had renal insufficiency at the time of biopsy had either normal or improved renal function at last follow-up.

The mean length of follow-up was significantly longer in NOS (46.3±4.7 months) compared to the other three groups. There was significantly greater use of steroids in GTL (97.2%) compared to all other groups and significantly less use of ACE/ARB in COLL (31.0%) compared to CELL (62.5%, P = 0.042) and GTL (66.7%, P = 0.006). There was significantly less use of calcineurin inhibitors in COLL compared to NOS but no significant difference in use of cytotoxic agents among the four FSGS subtypes. Mycophenolate mofetil was used in only two patients, both with CELL. GTL showed significantly higher CR + PR rate (75.8%) compared to CELL (44.4%, P = 0.027), COLL (13.2%; P < 0.001), and NOS (38.6%; P < 0.001), and significantly lower ESRD rate (5.7%) compared to all other groups. COLL showed the lowest overall remission rate (13.2%) and the highest rate of ESRD (65.3%). CELL showed intermediate PR and CR rate (44.4%) compared to COLL (13.2%, P = 0.009) and GTL (75.8%, P = 0.027) but not compared to NOS. CELL and COLL also showed significantly different rates of ESRD (27.8 vs 65.3%, P = 0.023). By Kaplan–Meier analysis, both

COLL and CELL showed significantly shorter time to development of ESRD compared to GTL and NOS; however, there was no significant difference between CELL and COLL.

Clinical–pathologic correlations in CELL and all FSGS cases

On average, black CELL patients were younger than non-black patients (mean age 28.9 ± 6.7 vs 53.7 ± 7.2 years, $P = 0.025$) and were less likely to show remission response than white patients ($P = 0.017$). Arteriosclerosis score correlated positively with patient age (linear function, $P = 0.008$) and with hypertension ($P = 0.018$). Serum creatinine correlated negatively with UVp (logarithmic function, $P = 0.035$, $r = 0.566$) and UVp correlated negatively with % segmental + global sclerosis ($P = 0.087$, $r = 0.441$), suggesting that higher protein excretion was not a function of more advanced glomerular disease. The presence of NOS lesions in CELL patients correlated with higher serum albumin (2.88 ± 0.39 mg/dl in five patients with NOS vs 1.71 ± 0.27 mg/dl in 17 patients without NOS, $P = 0.024$) suggesting that those without NOS were more nephrotic at presentation. However, there was no significant correlation between presence of NOS lesions and edema ($P = 0.055$), UVp, age, race, gender, serum creatinine, response to therapy, or rate of ESRD (all $P > 0.10$), or time to ESRD by Kaplan–Meier analysis.

For cases of CELL, the only correlate of any remission response (complete or partial) was white race ($P = 0.017$). Inverse correlates of ESRD included any remission response (CR/PR vs NR, $P = 0.036$) and duration of therapy ($P = 0.036$) suggesting that these patients had failed to respond to steroid therapy and that progression to ESRD was not due to failure to treat. Although five of 10 NR developed ESRD, compared to zero of eight CR/PR patients, these differences did not reach statistical significance by Fisher's exact test ($P = 0.06$), presumably reflecting the small sample size and the short follow-up. Similarly, although black patients were less likely to show remission response, it was not possible to demonstrate an association between black race and ESRD, reflecting the small number of events (one of seven black patients with ESRD versus four of 10 non-black patients, $P = 0.624$).

By multivariate analysis (Cox regression), no clinical or pathologic variable correlated with ESRD in CELL cases. However, when all FSGS patients were combined, predictors of ESRD included: initial serum creatinine (hazard ratio (HR) 1.270, confidence interval (CI) 1.192–1.354, $P < 0.001$); lack of any remission response (CR/PR vs NR) (HR 0.255, CI 0.131–0.495, $P < 0.001$); % global sclerosis (HR 1.025, CI 1.011–1.039, $P < 0.001$); % COLL lesions (HR 1.016, CI 1.002–1.031, $P = 0.029$); and chronic tubulo-interstitial injury score (HR 2.345, CI 1.514–3.633, $P < 0.001$). Age, race, gender, presence or absence of full nephrotic syndrome, UVp, serum albumin, and arteriosclerosis score were not predictive of progression to ESRD in the multivariate model for the whole FSGS population.

DISCUSSION

CELL FSGS, COLL FSGS, and GTL share clinical presenting features of heavier proteinuria, more frequent nephrotic syndrome, and shorter duration of symptoms compared to FSGS NOS, suggesting that these three morphologic variants reflect acute glomerular injury, or possibly a response to heavy proteinuria. However, these morphologic variants of idiopathic FSGS display significantly different rates of remission and ESRD that are worst for COLL, intermediate for CELL and NOS, and best for GTL. Among all FSGS patients, independent predictors of ESRD included initial serum creatinine, remission response, % glomeruli with COLL lesions and chronic tubulo-interstitial injury score, and % global sclerosis. These findings are consistent with the results of other studies, and support the recognition of COLL and GTL as distinct clinical–pathologic variants of FSGS. Although CELL displayed significant differences in renal outcome compared to COLL and GTL, this might reflect in part the inclusion of unsampled COLL and GTL in the CELL cohort, as suggested by the finding that CELL biopsies had significantly fewer glomeruli than COLL or GTL.

The initial description of CELL FSGS by Schwartz and Lewis[7] in 1985 did not exclude the presence of COLL features and these investigators do not distinguish CELL FSGS from COLL FSGS.[5,8] Indeed, two of our initial CELL cases proved to be COLL based on the finding of segmental capillary COLL on review of deeper levels. Further evidence that CELL and COLL may be pathogenetically related includes the demonstration of glomerular epithelial cell proliferation associated with evidence of a dysregulated podocyte phenotype, including altered expression of cell-cycle regulatory proteins, in cases of CELL, HIV nephropathy, and idiopathic COLL.[9] These findings raise the important question of whether CELL should be distinguished from COLL. However, in the present study we identified significantly different rates of remission and ESRD when CELL and COLL were classified as distinct morphologic variants using the Columbia FSGS Classification. Moreover, % glomeruli with COLL lesions was an independent predictor of ESRD in all cases of FSGS, emphasizing the negative prognostic impact of COLL lesions. These findings support that CELL and COLL lesions should not be classified together and validates an approach to pathologic classification that distinguishes between them.

Perhaps the strongest argument for distinguishing CELL from COLL lesions is that many cases (12 of 38) of apparent CELL proved to be undersampled GTL after examining additional (deeper) tissue sections and applying stringent criteria to define FSGS variants. CELL and GTL showed very similar pathologic findings including % global sclerosis, % segmental lesions, % NOS lesions, tubulo-interstitial scarring, arteriosclerosis score, and % foot process effacement, underscoring the potential for confusion of these subtypes, particularly in limited biopsy specimens. CELL lesion without tip lesion features might represent a more advanced stage of GTL where the relationship to the proximal tubule is no

longer present. In support of this hypothesis is our finding, in a previous study of GTL, that increased frequency of peripheral lesions (most of which were cellular) correlated with higher initial serum creatinine and worse renal outcomes, including absence of remission and development of ESRD.[4] In the present study, however, GTL and CELL showed similar mean time to biopsy, initial serum creatinine, % global glomerulosclerosis, tubular atrophy/interstitial fibrosis score, and arteriosclerosis score, arguing against CELL and GTL being different stages of the same disease process. A minority of CELL cases also contained NOS lesions; other than the presence of lower serum albumin in CELL cases with NOS there was no significant difference in other clinical characteristics, including renal outcomes, compared to CELL cases without NOS lesions. Given the possibility that undersampling of either GTL or COLL could lead to misclassification as CELL, additional tissue sections are clearly warranted in all biopsies with features of apparent CELL.

CELL variant of idiopathic FSGS comprised only 3% of all cases of idiopathic FSGS reported from the University of North Carolina/Glomerular Disease Collaborative Network over a 19-year period.[2] In the present study, we selected cohorts of FSGS from different time periods, thus the frequency of CELL cannot be determined from the data presented. However, among 6487 consecutive native kidney biopsies examined between 2000 and 2004 inclusively (the period when most of our CELL cases were diagnosed), a total of 716 cases were diagnosed with FSGS of which 446 (62.3%) were NOS or PH, 170 (23.7%) were COLL, 67 (9.4%) were GTL, and only 33 (4.6%) were CELL. Thus, CELL is clearly the least common morphologic variant of FSGS.

The pathogenesis of primary CELL is unknown but a key role for podocyte injury is evidenced by the findings of diffuse foot process effacement and glomerular epithelial cell hypertrophy/hyperplasia in most cases. In the renal allograft, recurrent FSGS often has CELL or COLL features,[10] and this has been linked to the presence of a circulating permeability factor in some cases.[11] However, the nature of this factor and its role in CELL in the native kidney has not been determined. The segmental lesions in CELL show variable features of endocapillary hypercellularity related to accumulation of inflammatory cells (mostly foamy macrophages, with or without neutrophils and other mononuclear cells). Similar findings are seen in GTL and some case of COLL, all of which are associated with heavy proteinuria, as well as in other human and experimental diseases characterized by proteinuria,[12] suggesting that the intracapillary hypercellularity might represent a localized inflammatory response to high transcapillary flux of a protein- and lipid-rich filtrate. Of note, the lack of correlation of CELL and GTL with serum cholesterol levels argues against hypercholesterolemia *per se* being a major pathogenetic factor in the morphogenesis of these lesions, although the role of other lipids is unknown. The higher ESRD rate in COLL may reflect the higher initial serum creatinine in this subgroup, as demonstrated by Cox regression analysis. In addition, in the present study COLL showed the highest degree of pathologic injury, including (% global + segmental lesions) and tubulo-interstitial scarring, despite shorter mean time to biopsy, suggesting an innately more aggressive disease rather than delay in recognition. These differences in ESRD rate also raise the question whether cellular and tip lesions may represent pre-sclerotic or even potentially reversible segmental lesions.

The prognostic value of morphologic classification of FSGS is not universally acknowledged,[13] reflecting the inherent difficulty of accurately classifying focal patterns of injury based on pathologic examination of limited biopsy samples, and the potential for different types of lesions to coexist in individual biopsy samples. Even in larger samples, the findings of a single glomerulus with diagnostic lesions of COLL or GTL may have markedly different implications for renal prognosis, underscoring the inherent problem of accurately classifying glomerular diseases that produce focal and segmental pathologic alterations. Nonetheless, given the findings of major difference in renal outcomes between COLL and GTL identified in this and other studies, we believe it is important to recognize CELL as a distinct morphologic lesion (defined by segmental expansion of the glomerular tuft with endocapillary hypercellularity, without features of COLL or GTL). Classification of all CELL cases as COLL would inevitably lead to inclusion of some cases of GTL where the defining tip lesion was not sampled, thereby diluting the prognostic significance of both COLL and GTL.

In summary, CELL identifies patients with idiopathic FSGS who present with short duration of heavy proteinuria/nephrotic syndrome, which, if unremitting, may lead to ESRD. As such, CELL lesions represent a feature of active FSGS. On deeper sectioning, some cases of CELL variant prove to be GTL or, less commonly, COLL variants. These sampling problems may explain in part the intermediate outcome of CELL relative to COLL and GTL. Despite these limitations of sampling, our data strongly support the view that CELL and COLL lesions are not equivalent. The different clinical features and prognostic implications of COLL, CELL, and GTL validate an approach to pathologic classification of FSGS that distinguishes between these morphologic subtypes.

MATERIALS AND METHODS

Case selection

A database search of all native kidney biopsies that were accessioned at the Columbia University Medical Center Renal Pathology Laboratory between 1998 and 2005 (inclusively) identified 48 cases that were originally diagnosed as CELL. Six cases of probable secondary FSGS were excluded, including three cases of hypertensive arterionephrosclerosis, one associated with morbid obesity, one with renal atheroembolic disease, and one case associated with solitary kidney. Three cases that showed immunofluorescence microscopy and EM features of C1q nephropathy, and one case that had less than five glomeruli by combined light and EM were also excluded. To rule out undersampled COLL or GTL, additional tissue sections were obtained on each of the remaining 38 cases. The pathologic

findings were jointly re-reviewed by three renal pathologists using a multiheaded optical microscope to arrive at a consensus diagnosis. Sixteen of these 38 cases were re-classified as GTL ($n=12$), COLL ($n=2$), or NOS ($n=2$) based on the recently published Columbia Classification scheme (see below) and examination of deeper tissue sections. For comparison of demographic and clinical presenting features with the 22 CELL cases, three cohorts were selected, including 56 cases of COLL (diagnosed between 1979 and 2001), 60 cases of GTL (diagnosed between 1998 and 2004), and 87 cases of NOS (diagnosed between 1979 and 2000). Details of some of these cases have been reported previously.[3,4] In the majority of cases, glass slides were available for re-review, including 46 cases of COLL, 49 cases of GTL, and 36 cases of NOS, and the morphologic classification of FSGS subtype in accordance with the Columbia FSGS classification scheme was confirmed. Only cases with >5 glomeruli per tissue section were included. In all cases, known secondary causes of FSGS were excluded, including HIV infection, intravenous drug use, vesico-ureteral reflux, chronic glomerulonephritis, morbid obesity, and familial renal disease. We did not include the PH subgroup because many of these cases represent secondary forms of FSGS owing to adaptive responses and the focus of this study was the comparison of pathologic variants of idiopathic FSGS. The Institutional Review Board of Columbia University Medical Center approved this study.

Clinical characteristics of FSGS patients

Clinical data were obtained from physician referral forms submitted at the time of biopsy and from follow-up telephone conversations. For adults ($\geqslant 16$ years of age), the following clinical definitions were used: NRP, $\geqslant 3.5$ g/day; hypoalbuminemia, serum albumin <3.5 g/dl; hypercholesterolemia, serum cholesterol >200 mg/dl; and renal insufficiency, serum creatinine >1.2 mg/dl. Nephrotic syndrome was defined as two or more findings of NRP, hypoalbuminemia, and peripheral edema. Hypertension was defined as systolic blood pressure >140 mmHg or diastolic blood pressure >90 mmHg, or receiving antihypertensive medications. For subjects aged $\leqslant 16$ years at initial presentation, NRP was defined as >40 mg/m^2/h;[14] hypoalbuminemia was defined as serum albumin <2.5 g/dl,[14] renal insufficiency was defined by a calculated creatinine clearance of less than 90 cm^3/min,[15] and hypertension was defined as a systolic or diastolic blood pressure $>$the 90th percentile based on the child's gender, age, and height percentile.[16] ACE/ARB were considered antihypertensive medications only where used specifically for the treatment of hypertension, not proteinuria. For outcome analysis, stable renal function was defined as a change in serum creatinine of $\leqslant 20\%$ of the initial value. CR was defined as UVp <0.3 g/day, random urine protein/creatinine ratio of $\leqslant 0.3$, or dipstick trace protein, and stable renal function at last follow-up. PR was defined as UVp of 0.3–2 g/day, or urinary protein/creatinine ratio of 0.3–2, with at least 50% reduction in proteinuria from initial presentation, and stable renal function at last follow-up. NR was defined as persistent heavy proteinuria (>2 g/day, or urinary protein/creatinine ratio >2) and/or progressive renal failure at last follow-up. ESRD was defined as the time point when renal replacement therapy was started.

Pathologic classification of FSGS

Renal biopsies were processed for light microscopy, immunofluorescence microscopy and EM according to standard techniques. For light microscopy, at least 11 glass slides, each containing 2–3 tissue sections (3 μm thick), were stained with hematoxylin and eosin, periodic acid-Schiff (PAS), Masson's trichrome and Jones silver methenamine stains. To minimize undersampling of COLL lesions or GTL, additional sections were obtained for all CELL cases (one PAS slide with 3–6 tissue sections per case). Routine immunofluorescence microscopy was performed on 3 μm thick cryostat sections using polyclonal fluorescein isothiocyanate-conjugated antibodies to IgG, IgM, IgA, C3, C1q, κ, λ, fibrinogen, and albumin (Dako Corporation, Carpinteria, CA, USA). EM was performed using a JEOL 100S electron microscope.

The percentage of globally sclerotic glomeruli and segmental lesions in each biopsy was calculated. Each lesion was categorized according to the Columbia FSGS Classification and each case was subsequently assigned to one of the FSGS variants.[1] COLL lesion was defined by the presence of either segmental or global glomerular capillary wall COLL with hypertrophy and hyperplasia of overlying podocytes (Figure 4). Tip lesion was defined by the presence of a segmental lesion involving the tip domain (the outer 25% of the tuft next to the origin of the proximal tubule), in which the tubular pole was identified and there was either an adhesion or confluence of podocytes with parietal or tubular epithelial cells at the tubular lumen or neck (Figure 5). CELL lesion was defined as occlusion of capillary lumina by segmental endocapillary hypercellularity (Figure 1), with or without foam cells, hyalinosis, and karyorrhexis, but without features of segmental COLL or tip lesion in this or any other glomerulus in the biopsy. CELL lesions could show podocyte hyperplasia and hypertrophy (Figure 1) but this feature was not required for diagnosis. CELL lesions could be located adjacent to the hilus (PH) or in the tuft periphery (peripheral). In all cases where peripheral CELL lesions were identified, adjacent tissue sections were carefully screened to exclude GTL. PH FSGS lesions consisted of hyalinosis or sclerosis contiguous with glomerular hilus (Figure 6), whereas NOS lesions consisted of segmental increase in matrix obliterating the capillary lumina, without features of COLL, tip lesion, endocapillary hypercellularity, or PH localization (Figure 7).

COLL variant FSGS was diagnosed if at least one glomerulus showed a segmental or global COLL lesion, regardless of findings in the remaining glomeruli. GTL was diagnosed if tip lesions were found, without evidence of COLL features in any glomerulus. CELL variant FSGS contained CELL lesions that lacked features of COLL or GTL whereas NOS variant was diagnosed by the presence of an NOS lesion in at least one glomerulus, after exclusion of COLL, GTL, or CELL. Of note, no case in this study showed features of PH variant FSGS ($>50\%$ PH segmental lesions, after excluding cases of COLL, GTL, CELL).

For CELL cases, the location of each segmental lesion with respect to the glomerular hilus and tubular pole was determined by tracking through adjacent tissue sections. The degree of podocyte hyperplasia and hypertrophy in each segmental lesion was graded semiquantitatively (mild, moderate, or severe) and the presence or absence of foam cells, mononuclear cells, and neutrophils were noted. Acute tubular injury was defined by the presence of tubular simplification, loss of brush border, and enlarged reparative nuclei with nucleoli, with or without mitotic figures. Tubular atrophy, interstitial fibrosis, interstitial edema, interstitial inflammation, and acute tubular injury were each graded semiquantitatively on a scale of 0–3+ based on the % cortical area affected (absent, $<25\%$, 26–50%, and $>50\%$, respectively). Arteriosclerosis and arteriolosclerosis were graded 0–3+ (absent, mild, moderate and severe, respectively) based on the degree of luminal narrowing and vessel wall thickening. The intensity of immunofluorescence staining was graded on a scale of

0–3+. The percentage of foot process effacement was determined quantitatively by estimation (without using morphometric measurements) based on examination of the ultrastructure of all non-sclerotic glomerular capillaries in all fields studied.

Statistical analysis

Statistical analysis was performed using non-parametric exact statistical methods, including the Fisher exact test (for categorical variables), the Wilcoxon rank sum (or Mann–Whitney U) test (for continuous variables between two groups), the Kruskal–Wallis and the Jonckheere–Terpstra test (for continuous variables among 3+ groups). Where parametric testing could be applied, comparison among multiple groups was performed by one-way analysis of variance. A standard test for homogeneity of variance was used to guide *post hoc* analysis (for assumed equal variance: the Tukey and Bonferroni tests and for unequal variance: the Tamhane and Dunnett tests). Bivariate comparison of two continuous variables was performed by standard curve estimation/fitting methods. ESRD-free survival time was calculated by the method of Kaplan and Meier and statistical comparison was made using the log-rank test. The Cox proportional hazard model was used for multivariate analysis of predictors of progression to ESRD. Analysis was performed using SPSS 14.0 for Windows (SPSS Inc., Chicago, IL, USA) and StatXact 6.0 for Windows (Cytel Inc., Cambridge, MA, USA). Continuous variables are reported as the mean $\pm$ s.e.m. Statistical significance was assumed at $P<0.05$.

ACKNOWLEDGMENTS

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